IDCPA Research Program
Chase-Recapture Experiment Consultation
Southwest Fisheries Science Center, La Jolla, CA
25-26 April 2000

Summary

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Background and Objective: This meeting is part of a series of consultations held by the Southwest Fisheries Science Center (SWFSC) with the Marine Mammal Commission (MMC) and Inter-American Tropical Tuna Commission (IATTC) regarding the required research projects identified in the International Dolphin Conservation Program Act (IDCPA). The IDCPA, a 1997 amendment to the Marine Mammal Protection Act (MMPA), mandates that the Secretary of the Department of Commerce, the parent agency of the National Marine Fisheries Service (NMFS), determine "whether the intentional deployment on or encirclement of dolphins with purse seine nets is having a significant adverse impact on any depleted stock in the eastern tropical Pacific Ocean." The IDCPA specifies three stress-related research projects including "an experiment

involving the repeated chasing and capturing of dolphins by means of intentional encirclement."

Anticipating the IDCPA, the SWFSC held a workshop in 1997 to identify potential methods for measuring fishery-induced stress on dolphins in the eastern tropical Pacific (ETP) (Curry and Edwards 1998). Invited participants at that workshop included academic scientists with expertise in stress in aquatic and/or terrestrial mammals and NMFS and IATTC scientists interested in determining whether purse seine fishing methods were causing physiological stress to dolphins in the ETP. The workshop participants recommended two basic approaches: 1) collecting samples immediately post-mortem from animals killed in the fishery (a necropsy program) and 2) collecting a time series of blood samples from repeatedly chased, captured, and released dolphins in order to follow a time course of various stress markers in the blood. (A necropsy program has been initiated in the form of a 'pilot program' consisting of samples from ten purse seine vessel fishing trips to determine if the sample collection was practicable and the sample quality satisfactory for laboratory analyses). As originally proposed, the chase-recapture experiment would provide physiological samples from live dolphins to complement the necropsy program, which would provide tissue and morphological samples from dolphins killed by the fishery.

The consultation summarized here sought to evaluate the potential of a chase-recapture experiment, one of the research projects discussed at the 1997 workshop, to provide data suitable for making the population level conclusions that are required under the IDCPA. Modifications to the suggested chase-recapture experiment and additional studies were discussed.

Barbara Taylor chaired the meeting and Meghan Donahue served as rapporteur. The following summary of the proceedings is structured on the agenda found in Appendix 1.

I. Introduction

After providing a general summary of the dolphins, the environment, and the yellowfin tuna fishery in the ETP, NMFS scientists summarized the research specified under the IDCPA and reviewed the chase-recapture experiment originally proposed during the 1997 stress research planning workshop (Curry and Edwards 1998). The main objectives of the 1997 workshop were to summarize the current state of research on stress in non-human mammals and to determine the most appropriate techniques to investigate the potential for fishery-induced physiological stress response in ETP dolphins. Specifically, the workshop aimed to identify physiological and morphological indices that could be used to reliably indicate the effects of stress in free-ranging dolphins involved in the tuna fishery. The participants included veterinarians specializing in marine mammals and experts on stress physiology, energetics, pathology, reproductive endocrinology and the ETP tuna purse seine fishery. The chase-recapture experiment as outlined at the 1997 workshop was suggested as a way to analyze the blood chemistry of

samples collected from live dolphins during repeated chase and encirclement. The idea was that a series of measurements from complete blood panels from captured dolphins could quantify the effects of chase and recapture and could give an indication of the trend in responses among a number of individuals with repeated exposure over time. Dolphins would be chased and encircled as they are in purse seine fishing operations and blood would be collected from a subset of animals and radio tags attached to still fewer. The radio-tagged dolphins would be tracked, recaptured after some number of days, and blood sample collection repeated. Serial sampling from the same individuals was deemed essential to identifying the trend of these measurements and would indicate whether or not recovery occurred between the repeated chase and capture events.

The strategy suggested was to evaluate the physiological markers measured from serial blood samples and analyze the series of data points for changes. Changes in these physiological markers would be used to examine the range of physiological responses and, if possible, to characterize physical condition (*i.e.*, "normal" or "potential fatality"). If normal, recovery would be considered likely for the animal. If the indications were poor, the animal would be classified as a likely fatality.

II. Discussion of experimental design: Population level inferences from relatively few individual measurements and other limitations.

It was recognized that the participants in the 1997 workshop were specifically aiming to identify physiological and morphological indices that can be practically measured to quantify the effects of stress in ETP dolphins and that they were not attempting to produce a thorough experimental design. The following concerns were discussed regarding the chase-recapture experiment as originally contemplated in the 1997 workshop .

Population level effects and sample size. There was general concern that the chase-recapture experiment, as originally proposed and perhaps in any logistically feasible variant, could not produce results that would lead to population-level inferences about effects of chase and capture on depleted dolphin stocks. That is, it is unlikely that information obtained from the few animals that could be sampled, given the types of things that could be measured, would be sufficient to extrapolate to entire affected populations. The experiment could sample at most about 10 or 15 dolphins, whereas the minimal number required for statistical validity would be about 300 per stock. (Sample size concerns are discussed further below. Required sample size computations and assumptions are given in Appendix 2). A counter view was advanced by Dr Geraci that although these measurements were unlikely to lead to statistical inference, they could be useful qualitative indicators. Serial sampling of dolphins after multiple chases and recaptures could identify animals with grossly-anomalous levels in blood parameters, and give an indication of the increases in levels occurring with repeated chases and captures. A large body of information for mammals in general could be used to indicate if such grossly-anomalous levels will likely lead to serious tissue damage or even death.

Sample representativeness and variability. Concerns were raised about the nature of the samples obtained, namely, the representativeness of the sample with respect to the entire population and likely variability of data from those samples. The representativeness of the overall sample is affected by the variability in blood measures within and between animals and the variability of the types of dolphins sampled. Because some of the factors measured in the blood complex can be quite variable, it is important to look at the entire complex so that the variability within and between individuals can be better addressed. Ensuring that the samples are representative in terms of factors such as age and sex is logistically challenging and the study would have to rely on extrapolation to address this issue. In addition, individuals of the same age and sex may respond differently to stress; some animals might be deleteriously affected by the stress of a chase and encirclement event whereas others may not . It was noted that animals that experience the greatest effect (*i.e.,* those that become moribund) may not be recaptured and will not be taken into account in the analysis. Similarly, false negatives may also occur when an incorrect prognosis for survival is made for an individual animal based on collected blood samples. Also, if stress effects are cumulative over a period longer than the field study, the experiment suffers from not knowing the history of the individuals being sampled.

It was also noted that sample captures may not be random in relation to history of exposure to the fishery. That is, animals more experienced with the fishery may have undergone less stress but may be more difficult to capture, whereas less-experienced animals that may be more affected by the event of the sample capture also may be easier to capture and sample. Many concurred that such a bias may indeed exist and the direction of that bias would need to be known, but couldn't be determined given current information.

Furthermore, it was also noted that sampling of calves was unlikely to be part of the final experimental design primarily because of permit restrictions under the MMPA.

Lack of Controls. A serious limitation of this experiment was identified as the lack of controls. That is, no practical method has been proposed to obtain observations from unstressed, un-chased or captured individuals of the target stocks. Like the original Workshop Group, this group acknowledged that permission could not be obtained for the terminal sampling that conceivably could provide a basis for comparison with samples from ETP dolphins. As a result, no adequate control samples could be collected. A related issue is the possibility that physical handling of animals during blood collection may induce a response not normally associated with chase and encirclement during regular fishing operations. Because animals must be handled to obtain blood samples, this potentially confounding factor is introduced and may produce false positives. Although some comparative analyses with stranded or captive dolphins may be useful, the group was not confident that this factor could be separated out of the analyses sufficiently. Because baseline values for the blood parameters are not available, the experimental design involves the initial sample of a series serving as the baseline. A liberal range would be applied to this baseline and levels exceeding that range would be used to draw

conclusions about individual animals. Although the group did not specifically discuss the suitability of scaling data from other species (if available) it was noted that some of the physiological markers measured here are not considered variable among species and that when those markers exceed a certain level the effect is pathological and injurious regardless of species.

Narrow scope. The chase-recapture experiment was considered by the group to be overly narrow. The population biologists present regarded it as very unlikely that any inferences could be made regarding reproductive parameters, given the sampling restrictions for this experiment. However, many considered effects on reproduction to be the most likely route for repeated chases to have a population level effect. Furthermore, if effects of stress are cumulative, we will not know enough about the history of individuals captured to assess this potential source of stress. Because the chase-recapture experiment does not address reproductive effects, a finding of 'no effect' would still provide no data on perhaps the most likely demographic factors to be affected.

Attribution of results. Many participants were concerned as to how the observed pathology as indicated by the blood parameters can confidently be associated with the chase and encirclement event and that attribution of cause would be problematic given current diagnostic tools and logistical and analytical constraints.

Several of these concerns specifically arose during a discussion about the frequency of the effect and the probability of detecting that effect. These considerations are summarized in Appendix 2.

III. Discussion of whether the chase-recapture experiment can fulfill the IDCPA requirements.

After discussing the limitations of the experiment as suggested, the group explored additional elements or modifications to the experiment that might allow population level effects to be addressed. The group was reminded that the chase-recapture experiment is one of a suite of studies being conducted under the IDCPA that includes abundance surveys, ecosystem studies, and various studies addressing fishery effects. A decision analysis framework is being developed in which the results of all of these studies can be quantitatively integrated. The planning workshop for stress-related studies, during which the idea for the chase-recapture experiment was developed, occurred prior to the consultations in which the decision analysis framework was formulated. Thus, an experimental design that would produce results that can be quantitatively integrated into the decision analysis framework was not considered at the time the experiment was conceived.

Given the original intent and design of the chase-recapture experiment, the group discussed whether measurements from the limited number of individuals sampled during the chase-recapture experiment could be used to make population level inferences and whether the experiment could be modified to better address the population level assessment required under the IDCPA. It was widely accepted that the chase-recapture

experiment, by addressing physiological indicators in a limited number of animals, could not provide sufficiently quantitative results or acceptable statistical power based on the limitations summarized above. Thus, a consensus was reached that the experiment as originally proposed was not capable of answering the specific question posed by the IDCPA.

IV. Alternative approaches, experiment modifications, and additional studies .

The group discussed a range of alternative chase-recapture experiments and additional studies that could be pursued. No one experiment, however, could provide answers on population level effects due to stress-induced mortality, stress-induced reproductive interference, and chase-induced loss of calves. The additional experiments and modifications to existing experiments involved field work, laboratory work and modeling exercises and addressed various areas such as stress indicators, mortality effects, fecundity/lactation and behavior/social disruption.

Suggested modifications or additions to field work involving "repeated chasing and recapture" of dolphins:

1. Estimate cow-calf separation and dropout by incorporating radio tagging and possibly recapture. Work already completed by the SWFSC establishes that cow-calf separation is a concern. The objective here would be to obtain a direct minimum estimate of this separation. This project narrowly focuses on a topic that is not stress-related, but still a potential cause of mortality. It addresses mortality that would result when animals drop out of the chase. As noted by Kilpatrick and Gerrodette (see also Appendix 2), this proposal suffers from the same sample size problem as the original study. It was suggested that it does not suffer from the false positive problem, the false negative problem can be controlled, and it addresses the issue of calf mortality. However, it was noted that potential biases could be present if the presence of an untagged calf with its tagged mother cannot reliably be detected by observers on board the helicopter or in aerial photographs. For example, given the possibilities of 1) missed calf detection caused by rough seas or helicopter drift, and 2) survival of a calf even after separation, a false positive result could occur. Likewise, given the possibility of misidentifying a tagged, separated cow with a calf not its own (in a tight group of cows and calves), false negative results could also occur.

   One suggestion was made to simplify the experiment by not actually re-capturing the school but just following animals, especially cows with calves, during a chase (from a helicopter or other means) to determine if and when they drop out. Some participants with much ETP field experience felt collecting this observational data would be too difficult from a helicopter or other platforms, especially because schools tend to scatter upon being chased

and individual animals, particularly calves, are difficult to see or follow for any length of time.

Detection of vocalizations during chase and encirclement, especially detection and quantification of calf separation, was suggested as an adjunct to the cow-calf separation experiment described above. However, NMFS scientists who conduct acoustics research were not confident that such a study was currently feasible.

2. Tag animals using satellite tags with added data loggers that measure time, depth, and velocity to address capture rate and to characterize an individual animal's experience in fishing operations (*e.g.*, speed and duration of chase for an individual animal). Data addressing these elements could probably only be collected from a small number of animals, but if the satellite tracking experiment was coupled with shorter-term radio tracking and observational studies, the satellite data transmission could be used to assess the longer-term chase history of that animal. It was also suggested that the satellite tags be equipped with heat-flux disks and thermocouple leads to simultaneously assess the thermal status of the individual (these data could be incorporated into the study of thermal stress in chased and encircled dolphins being conducted by Pabst and her colleagues under contract with the SWFSC. It was added that comparative studies with captured and captive dolphins would strengthen the interpretations of these thermal data. In addition, energetics and thermal stress information could also be explored from the velocity data. Modeling energetic costs of chase and encirclement was considered potentially fruitful and could be done in conjunction with data collected from these satellite tags in the field [see related study suggested in number 4 under 'additional studies (field and laboratory)'].

3. Tag animals using PIT technology to characterize dolphin movement and the capture experience. Although PIT tag technology has improved significantly and PIT tags have the advantage of being nearly permanent, past experience with implanting objects under delphinid skin raises serious concern that the tags may migrate out of the animals. Deployment and detection logistics would also be complicated and challenging. Extensive cooperation of the fishing fleet would be required, the detection distance (approx. 18 inches) is short, and the time frame required to obtain results is much longer than the remaining duration of the IDCPA research program.

4. Pursue thermal stress studies further to investigate whether dolphins are experiencing hyperthermia and its associated effects during chase and encirclement, with the primary focus being on the potential effect of thermal stress on reproduction. This group of studies would include: 1) collecting infrared thermal images of surface temperatures of dolphins under different conditions (resting, swimming at normal cruising speeds, bow-riding and

during chase by the research vessel) to identify the range of surface temperatures and the pattern of heat dumping and to compare temperature profiles of chased dolphins with non-chased dolphins; and 2) collecting deep core temperatures to assess the thermal status of individuals, which would provide evidence of deleterious thermal effects of chase and capture. These studies could include assessing temperature effects through elevated core temperatures that may effect reproductive fitness and deleterious effects on fetuses that may reduce their viability. This study is capable of generating a large sample size using a thermal camera that can collect data in a non-invasive manner. This approach would require ground-truthing experiments with ETP dolphins and/or closely-related animals.

Additional studies (field and laboratory) and modifications to existing laboratory studies:

1. Using contemporary and historical data, expand the tissue/skin studies addressing chronic stress to include pregnancy and lactation questions and spatial correlations to compare stress profiles between various areas of the ETP with differing histories of purse-seine fishing.
2. Examine preserved dolphin fetuses to determine if deleterious themal effects on fetal development are apparent. The initial goal would be to evaluate the potential of archived samples for a study of growth, body composition and developmental health. The SWFSC does have approximately 400 preserved fetuses from the ETP, however, the majority of these are smaller than 25 cm in length. It was suggested that both the composition of the archive (*i.e.*, most fetuses less than 25 cm in length) and the small size of the archive relative to the effect we are trying to detect may be problematic for such a study.
3. Large-scale tagging study to estimate mortality. Large-scale tagging studies have been attempted for ETP dolphins before with limited success. The recovery rate was deemed far too low and the logistics and timing were not considered feasible on the large scale necessary for meaningful results.
4. Estimate calf mortality by completing the genetic matching of cows to calves from historical sample archives. (This study is currently underway at the SWFSC).
5. Model the energetic cost of chase and capture and assess energy required based on the average number of sets per year under various scenarios (e.g., heavily fished versus less-fished areas, etc.). A model could also be explored for cow and calf energetics and swim speeds to investigate such issues as the ability for calves to maintain association with the cow during chase and the potential for interruption or postponement of reproduction or lactation for the cow because of energetic costs of chase and capture. Current information on the muscle morphology and histochemistry of young dolphins could be valuable to this exercise and to developing the hypothesis that chase could cause separation of cows and calves.

6. Reinstate life history sampling in fishery. The group was pessimistic that the necessary cooperation and logistics could be achieved in the time frame at hand but acknowledged the past and future value of such data in addressing the tuna-dolphin problem over the longer term.
7. Study physiological measures of captive animals to better link measured responses in samples from wild animals with their prognoses for survival. Such captive studies would almost certainly have to be done on delphinid species other than spotted or spinner dolphins, because these two species have proven extremely difficult to keep alive in captivity. These among-species differences in ability to survive capture and handling would of course confound results of the captive studies, at least in part.
8. Collecting biopsy samples that include skin, blubber and some muscle to determine, among other things, social structure (relatedness within groups) and possibly pregnancy rates. It was argued that pregnancy state could be elucidated from skin and blubber from standard biopsy samples and that developing a tool to sample muscle as well was thought to be too invasive and require too much technological development.

Blood sampling as originally proposed was considered by all to be an important part of any field effort because it can offer unique insight into the physiological status of the animals. Although these data may not be scalable to a population level, they may be useful in discovering a stress effect. Some participants questioned whether serial blood sampling, which would require the tracking and recapturing of individual animals, was necessary and suggested that non-serial blood sampling in conjunction with some of the other experiments proposed may be just as valuable. Because the group did not attempt to finalize an experimental design, they did not recommend one type of blood sampling over the other. It was noted that not all the experiments proposed here that could comprise a chase-recapture experiment would be compatible. Field methods and sample sizes of each study will impose constraints on the experimental design of the chase-recapture experiment that this group did not attempt to reconcile. In addition to the chase-recapture experiment, many participants also recommended the continuation of necropsy tissue sampling and analysis. This study has the potential to evaluate stress related to lesions in tissue such as heart muscle.

Because it was noted that many of these suggested studies required more research and development time than is available before completion of the IDCPA research program, many recommended including in the final research report a summary and prioritization of the valuable, longer term projects that could be pursued and are necessary to more fully resolve the question posed by the IDCPA.

Many participants agreed that a chase-recapture experiment could make a stronger contribution to answering the question posed by the IDCPA if it were modified to include the cow-calf separation study, satellite tagging, and biopsy sampling, in addition to blood collection. The experiment would also gain strength by being coupled with further historical archive work and thermal stress investigations. The group did not attempt to

reach a consensus on all the listed suggestions, rather the list was accepted as a good menu that will be useful in further developing and executing a study that will contribute more strongly to the final IDCPA research program report submitted before the final finding in December 2002. A thorough critique of the proposed modifications and additions was not undertaken at this meeting. However, some limitations of these proposed projects were identified and in some cases were similar to those limitations noted in the chase-recapture experiment as previously developed (e.g., required sample size and ability to translate effects on individual animals to population effects).

In summary, the original chase-recapture experiment was considered insufficient because of five major shortcomings: (1) inadequate sample size; (2) problematic sample representativeness problematic (in particular, there is no sampling of calves); (3) lack of controls; (4) too narrow a scope, especially regarding reproductive effects of stress; and (5) inability to confidently attribute the physiological results of the experiment to chase and encirclement events. Participants felt that the alternative experiments, coupled with other research discussed, could provide a stronger contribution to addressing the issue of causality.

References:

Curry, B. E. and E.F. Edwards. 1998. Investigation of the potential influence of fishery-induced stress on dolphins in the eastern tropical Pacific: Research planning. NOAA Technical Memorandum NMFS-SWFSC-254. 59 pp.

Appendix 1. Agenda.

ASSESSING EFFECTS OF PHYSIOLOGICAL STRESS AT THE POPULATION LEVEL: DISCUSSION OF A PROPOSED CHASE/RECAPTURE EXPERIMENT

Radisson Hotel
9:00am-5:00pm
April 25-26, 2000
La Jolla, CA

Convened by:
International Dolphin Conservation Program Act Research Program
Protected Resources Division
Southwest Fisheries Science Center
National Marine Fisheries Service
La Jolla, CA 92038

MEETING OBJECTIVES

1) Discuss and evaluate potential for proposed experiment to provide data suitable for making population level inferences as required by the law (IDCPA)

2) Discuss alternatives
   a) other field research
   b) historical data analyses

3) Provide advice about favorable avenues for future research to address stress-related issues

AGENDA

I) Introduction
   - IDCPA Information (summary)
     - What the law says NMFS ("Significant adverse impact, populations")
   - ETP Information Review (summary)
     - Environment
     - Dolphins
     - Fishing Procedures
   - Research Constraints
   - Chase/Recapture Experiment
     - history of development
     - summary of proposed activities

II) Discussion of General Requirements:
   - Population Level Inference from relatively few individual measurements

III) Discussion of whether Chase/Recapture experiment can fulfill IDCPA requirements

IV) Discussion regarding other field research which might fulfill IDCPA requirements

V) Discussion of alternatives being explored currently

VI) Develop/suggest recommendations for future directions, given constraints of ETP research and timing of IDCPA requirements.

VII) Closing Remarks

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Appendix 2. Considerations of experimental design for detecting the unobserved mortality effect (submitted by Tim Gerrodette).

(1) Frequency of the effect. Based on the 1999 Report to Congress, the number of dolphins necessary to account for the difference between observed and expected population size is approximately μ_{98}, which is about 22,000 northeastern offshore spotted and 10,000 eastern spinner dolphins. Thus, we are looking for a mortality effect associated with chase and encirclement that would produce 32,000 deaths/year. Reported mortality is <3,000, so this presumed unobserved mortality effect is more than 10 times the reported kill rate. The total number of events (dolphins set on) that produce this number of deaths is approximately (8,000 sets/year) x (400 dolphins/set) = 3,200,000 dolphins/year. The number of 400 dolphins/set is a rough estimate obtained from the reported mean school sizes from tuna vessels (about 450 for spotted and 350 for spinner dolphins). Many dolphin sets occur on mixed spotted/spinner schools, so the total school size would be the sum of the number of spotted and spinner dolphins separately. Furthermore, these figures are the means for all schools seen, whereas fishermen tend to set on larger schools. On the other hand, the dolphin school may split, and not all dolphins in the school may be exposed to the full chase and encirclement procedure. Overall, the figure of 400 dolphins/set is probably a conservative (low) estimate. If all 32,000 deaths were due to a single cause such as muscle damage, the condition should be present, on average, in 32,000/3,200,000 = 0.01 of the animals on any particular set. It is more likely that several of the factors identified at the meeting as potential mortality effects are acting, so the fraction of animals that show any particular detrimental condition will be smaller. Therefore, in the following calculations it is assumed that we are trying to observe a lethal condition that is present in 0.001 to 0.01 of the animals.

(2) Statistical model. The probability of observing k events (observation of a dolphin with a lethal condition) in n trials (number of dolphins sampled) with event rate p (0.01, 0.001) is

$$Binomial(k;n,p) = \binom{n}{k} p^k (1-p)^{n-k}$$

The Poisson distribution $f(k) = \exp(-\lambda)\, \lambda^k / k!$ could also be used and in this case gives identical estimates of the probability of observing k events with mean λ ($=np$).

(3) Other assumptions. (a) It is always possible to make a clear determination that an animal does or does not have the lethal condition. If, as is likely, there is some ambiguity about assessing the condition of an animal, the rate at which an unambiguous condition is present will be less than the range assumed. (b) Dolphins are sampled randomly. If dolphins with a lethal condition have either a higher or lower probability of being sampled, then the effective event rate will be increased or decreased. If dolphins with lethal conditions displayed different behavior, it would be possible to concentrate sampling intentionally on those individuals in order to demonstrate the existence of lethal conditions, and thus to increase the probability of detection, but this procedure would preclude inference to the whole population.

(4) Probability of failure to detect. Previous discussion of a chase/recapture experiment considered a sample size of $n = 6$ animals from which enough repeated blood samples could be obtained in order to determine if a lethal condition exists or not. According to an estimate presented at this meeting, we could expect to obtain repeated blood samples from 24-32 dolphins during a cruise of 30 working days. The probability P of not observing a lethal condition in a sample of 6 animals is $P = \text{Bin}(0) = 0.94$ for $p = 0.01$ and $P > 0.99$ for $p = 0.001$. For a sample of 32 animals, the probabilities are 0.72 and 0.97. Thus, given the logistical and financial constraints of the situation, there is a high probability of failing to detect a lethal condition that is present at a level sufficient to prevent dolphin populations from recovering.

(5) Sample size. The number of dolphins n that must be sampled in order to observe, with 95% probability, at least one dolphin with a lethal condition is the minimum n such that $\text{Bin}(0) < 0.05$. Thus, $n = 300$ (for p=0.01) to 3,000 (for p=0.001) dolphins. These sample sizes are much larger than are possible in the proposed chase/recapture experiment.